SPECTRAL CALIBRATION OF FLUORESCENT POLYNUCLEOTIDE SEPARATION APPARATUS

Cross-Reference to Related Applications

This application is a continuation-in-part of U.S. Patent Application Serial No. 09/154,178 filed September 16, 1998, Pat No. 6 821 402 incorporated herein by reference.

Field of the Invention

The invention is in the field of spectral calibration of fluorescence based automated polynucleotide length measurement instruments.

Background

In fluorescence-based DNA analyzers, fluorescence spectra are acquired by exciting the sample during the analysis/assay. The information of interest, e.g., called bases or genotypes, is generated by transforming the fluorescence spectra acquired during analysis/assay to "dye amounts," *i.e.*, how much of each dye is present or being generated during the analysis/assay.

Consider, for example, the simple case of determining the amounts of two dyes present in a solution using spectral sensors. The fluorescence emission at each spectral sensor (wavelength region or CCD bin) is the sum of the contributions of each dye. This can be expressed mathematically as:

Signal at sensor i = Emission of Dye 1 at sensor i + Emission of Dye 2 at sensor i (I)

The first thing to note about equation (I) above is that it contains one known quantity (measured signal at sensor i), and two unknown quantities (the emission of each dye at sensor i). Since there is one equation having two unknowns, no unique solution can be found. It is important to note that including more sensors (for example a second sensor j) is not necessarily helpful because each sensor adds an equation similar to equation (I) with two unknown quantities, namely the contributions of the individual dyes to the signal acquired at